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(54) Element, method and kit for the determination of an analyte in a fluid

(57) The subject matter of the invention is an element for the determination of an analyte in a fluid by means of a specific binding reaction of two binding partners with biological affinity

containing in or on the material allowing transport of fluid between the zones a sample application zone (1) and a downstream detection zone (4)

and a zone (3) with an immobilised analyte or analyte analogue which is located between the sample application zone (1) and the detection zone (4)

and upstream zone (3) with an immobilised analyte or analyte analogue, conjugate 1 which is impregnated and can be removed by a fluid and consists of a binding partner 1, which has biological affinity and is able to form a specific binding reaction with the analyte to be determined, and a detectable marker 1,

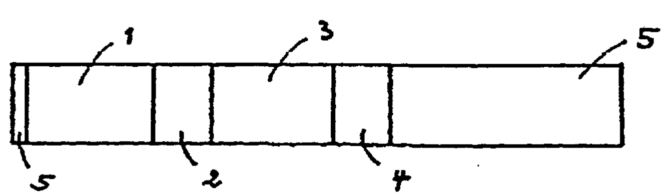
characterised in that

the detectable marker 1 is a low molecular organic molecule

and upstream zone (3) with an immobilised analyte or an analyte analogue, a universal conjugate 2 is present which, too, can be removed by a fluid and consists of a binding partner 2, which has biological affinity and is able to form a specific binding reaction with the detectable marker 1, and a visually detectable marker 2.

Moreover, the subject matter of the invention is the use of an element of the invention for the determination of an analyte and a kit for the determination of an analyte containing the element and elution agent of the invention.

Fig. 1



Description

[0001] The invention relates to an element for the determination of an analyte in a fluid by means of a specific binding reaction of two binding partners with biological affinity containing, in or on material allowing transport of fluid between zones, a sample application zone and a downstream detection zone as well as between the sample application zone and the detection zone, a zone with immobilised analyte or analyte anologue and in the sample application zone or upstream or downstream, a conjugate which is impregnated and can be removed by a fluid and which consists of a binding partner with biological affinity capable of a specific binding reaction to the analyte to be determined and a detectable marker.

[0002] Elements of that kind are known, for example, from DE-A3842702 or DE-A4439429. There, the analyte elements contain the reagents necessary for carrying out immunoenzymometric or immunoenzymometric-analogous determination methods. These are, in particular in a zone between the sample application zone and detection zone, immobilised analyte or analyte analogue and a conjugate consisting of a binding partner with biological affinity capable of a specific binding reaction to the analyte to be determined and a detectable marker.

[0003] In the case of DE-A 3842702, an enzyme marker is described as detectable marker. In order to make this marker visible, it is necessary to contact it with a chromogenic enzyme substrate so that a colour appears due to the enzymatic activity. The requirement to make the marker visible is not straightforward, expensive due to the steps to be taken and, moreover, optionally involves technical difficulties, for example when the corresponding enzyme substrate in the element for analysis has problems as to stability.

[0004] Therefore, in recent years, direct markers have been preferred, as described in DE-A 4439429. These direct markers are, for example, metal or latex particles which are colourful already und can be detected with the naked eye. Nowadays, gold marking is particularly preferred. For this purpose, depending on the analyte, a correspondingly marked binding partner with biological affinity is produced, for which optimum conditions for reaction and storage have to subsequently be created on the element for analysis. Each adjustment to the analyte to be determined requires a great effort. Depending on the analyte to be determined, the polyclonal or monoclonal antibodies necessary for this purpose can behave in very different ways during conjugation to the gold particles. This can lead to different stabilities of gold conjugates. Due to the different behaviour of

different polyclonal antibodies or different monoclonal antibodies during charging of gold particles, the spatial arrangement of the antibodies on the gold particles can be very different, which can cause steric problems during reaction of such conjugates with analyte and, thus, low sensibility.

[0005] It was, therefore, the problem of the present invention to avoid these disadvantages and to provide an element for analysis containing stable, simple and reproducible reagents which make a sensitive determination reaction possible.

[0006] This problem has been solved by the subject matter of the invention, as illustrated in the claims.

[0007] Subject matter of the invention is an element for the determination of an analyte in a fluid by means of a specific binding reaction of two binding partners with biological affinity

> containing in or on the material allowing the transport of fluid between zones a sample application zone (1) and a downstream detection zone (4)
>
> and a zone (3) with an immobilised analyte or analyte analogue which is located between the sample application zone (1) and the detection zone (4)
>
> and upstream the zone with an immobilised analyte or an analyte analogue, conjugate 1 which is impregnated and can be removed by a fluid and consists of a binding partner 1, which has biological affinity and is able to form a specific binding reaction with the analyte to be determined, and a detectable marker 1,
> characterised in that
>
> the detectable marker 1 is a low molecular organic molecule
>
> and upstream the zone with an immobilised analyte or an analyte analogue, a universal conjugate 2 is present which, too, can be removed by a fluid and consists of a binding partner 2, which has biological affinity and is able to form a specific binding reaction with the detectable marker 1, and a visually detectable marker 2.

[0008] A further, subject matter of the invention is the use of an element of the invention for the determination of an analyte and a corresponding determination method. This method for the determination of an analyte by means of an element according to the invention is characterised in that

the sample application zone is contacted with analyte,

the analyte is moved in the direction of the detection zone with a fluid,

the analyte present in the fluid reacts with conjugates 1 and 2 to form a detection complex,

the detection complex is transported to the detection zone with a fluid

and is determined in that zone.

[0009] Finally, a further subject matter of the invention is a kit for the determination of an analyte containing an element for analysis according to the invention and an elution agent.

[0010] The determination of an analyte by means of an element according to the invention takes place due to specific binding reactions between two binding partners with biological affinity. Binding partners with biological affinity and corresponding specific binding reactions between the binding partners are known to the person skilled in the art. Binding partners with biological affinity are, for example, hapten and antibody, antigen and antibody, lectin and sugar or saccharide, avidin or streptavindin and biotin as well as nucleic acid and nucleic acid, ligand and receptor. In this context, an antigen can be any molecule against which it is possible, experimentally, to produce antibodies. An antibody, too, or a specific site of an antibody designated epitope can be an antigen and be specifically detected and bound by an antibody. Nucleic acids relate to all possible forms of nucleic acids which are capable of binding via complementary bases. Specifically, but not as a final list, DNA, RNA, but also nucleic acid analogues, such as peptide nucleic acids (PNA, see, for example, in WO92/20702). Ligand and receptor generally relate to a specific binding interaction between two partners, such as, for example between hormone and hormone receptor.

[0011] In the element of the invention, there are the reagents necessary for carrying out the determination of an analyte as well as further zones necessary for the function of the element in or on material allowing transport of fluid. It is essential for the element for analysis according to the invention that the fluid within the element can move in the direction of the detection zone. Such flux of fluid is possible, for example, in a hollow body prepared accordingly by means of gravity. Devices allowing transport of fluid by centrifugal force as a form of gravity are known, for example from EP-B 0052769. However, elements for analysis according to the invention preferably contain absorbable materials capable of moving fluid by means of capillary force. In this context, the materials of the individual zones of the element according to the invention can be the same or different. Often it is the case that different zones consist of different materials if these are to carry out their task in an optimum manner.

[0012] In principle, possible absorbent capillary-active materials can be materials which generally can be used for absorbing fluid in so-called "dry tests", as described in US-A 4,861,711, US-A 5,591,645 or EP-A 029 1194 or in DE-A 3842702 or DE-A 4439429. For this purpose, for example porous materials such as membranes, for example nitrocellulose membranes, have proven to be advantageous. However, fibrous, absorbent matrix materials such as fleeces, tissues or knitted fabrics can be used, too. Fleeces are particularly preferred. Fibrous matrix materials can contain glass, cellulose, cellulose derivatives, polyester, polyamide, but also viscose, viscose staple fibre and polyvinyl alcohol. Fleeces of fibres on the basis of cellulose, polymer fibres on the basis of polyester and/or polyamide and an organic binding agent with OH- and/or ester groups as known from EP-B-0326135 can, for example, be used according to the invention. Fleece materials containing fusible copolyester fibres, apart from fibreglass, polyester fibres, polyamide fibres, cellulose fibres or cellulose derivative fibres as described in the European patent application 0571941 can also be used in the element for analysis according to the invention. Papers, as for example paper for tea bags, are also good to use.

[0013] To improve the way the elements for analysis according to the invention can be handled, the absorbent capillary-active material or different absorbent capillary-active materials can be arranged on a stiff carrier material which is not permeable for fluids, does not influence the flux of fluid in the matrix material in a negative way and behaves inertly as to the reactions taking place in the element for

analysis. Preferred carrier material can, for example, be polyester foil onto which the matrix material allowing for the transport of fluid is fixed.

[0014] In the element of the invention the individual zones can be positioned on top of each other, next to each other or partly on top of each other and partly next to each other on the carrier material. An element for analysis of the invention where the sample application zone, the zone with an immobilised analyte or analyte analogue and the detection zone are positioned next to each other on the carrier material is particularly preferred. In this connection, next to each other means that these zones are in direct contact with each other in the direction of the fluid transport or that they are separated by other zones and are localised essentially on one level.

[0015] The sample application zone is the area of the element of the invention onto which the sample in which it is to be determined whether a certain analyte is present, optionally in which amount it is present is applied.

[0016] The detection zone is the area of the element for analysis of the invention in which it is determined whether the analyte examined was present in the element of the applied sample. This determination can be qualitative, semi-quantitative or quantitative. In this context, semi-quantitative means that no concrete concentration value but a concentration range is determined for the analyte, in which the analyte concentration is located.

[0017] According to the invention, there is a zone with immobilised analyte or analyte analogue between the sample application zone and the detection zone. In this context, analyte analogue means a substance which reacts to the analyte to be detected in a comparable way with respect to the specific binding reaction with the binding partner with biological affinity.

[0018] The immobilisation of the analyte or of the analyte analogue on a matrix material between the sample application zone and the detection zone can be carried out according to methods known to the person skilled in the art. Thus, it is, for example, possible to adsorb the analyte or the analyte analogue onto the suitable matrix material in such a way that under test conditions there is no removal of the analyte or of the analyte analogue with fluid. It is, however, of course also

possible to carry out an immobilisation chemically by forming covalent bonds. An analyte can either be immobilised to the matrix material directly or via a spacer. In the case of a spacer, the analyte will, as a rule, be chemically modified by means of a corresponding spacer and then this analyte analogue will be bound to the matrix material. An indirect binding of the analyte or of the analyte analogue to the matrix material can, however, also be carried out via two binding partners with biological affinity like, for example, biotin and streptavidin.

[0019] In the case of the determination of haptens, polyhaptens have proved particularly suitable as analyte analogues. Polyhaptens are substances which have a plurality of haptens so that it is also possible to specifically bind a plurality of binding partners with biological affinity thereto. Due to the high density of binding partners with biological affinity which can be achieved in this manner, a high sensitivity of the test can be reached.

[0020] According to the invention, it is necessary that two conjugates are present on the element. These conjugates can be located on the sample application zone. They can, however, also be located upstream or downstream the sample application zone. The conjugates can be present impregnated in the corresponding matrix material. They can, however, also be coated onto the matrix material. Conjugates 1 and 2 can be present in the form of a mixture. They can, however, also be located separately and, in this case, they do not necessarily be directly next to each other. In the latter case, it is then also possible that there is, for example, one conjugate upstream the sample application zone and the other conjugate is, for example, in the zone between the sample application zone and the zone with an immobilised analyte or analyte analogue.

[0021] Conjugate 1 consists of a binding partner 1 with biological affinity which is capable of forming a specific binding reaction with the analyte to be determined and of a detectable marker 1. For the case that the analyte to be determined is a hapten or an antigen, the binding partner 1 with biological affinity will be an antibody which can form a specific binding reaction with the analyte. If the analyte is an antibody, the binding partner 1 with biological activity can be a corresponding hapten or antigen which forms a specific binding reaction with the antibody.

[0022] In accordance with the invention, the detectable marker 1 means a low-molecular organic molecule, preferably an organic molecule with a molecular weight smaller than 1,500, more particularly preferred smaller than 1,000, which, as a hapten, can be bound by a corresponding antibody in a specific binding reaction and which can be detected in this manner. For this purpose, digoxigenin or digoxin have proven to be excellently suitable. Digoxin is particularly preferred.

[0023] Marker 1 is preferably covalently bound to the binding partner 1 with biological affinity. Such conjugates can be reproducibly manufactured by means of simple organic chemical reactions.

[0024] Conjugate 2 is, independently from the analyte to be detected, a universally usable conjugate consisting of a binding partner 2 with biological affinity which can form a specific binding reaction with the detectable marker 1 and of a visually detectable marker 2. A particularly preferred binding partner 2 with biological affinity is an antibody against the low molecular organic molecule which is used as marker 1. So-called direct labels, i.e. markers which can be detected with the naked eye due to their colour without further process steps, are preferably used as visually detectable marker 2. Advantageous markers of this kind are, for example, particles which are insoluble in water like metal or latex particles but also pigments such as silicate, carbon black or selenium. According to the invention, metal particles, in particular, are preferably used as markers. Colloidal gold is particularly preferred as marker. Marker 2 can be bound covalently or also by adsorption to binding partner 2 with biological affinity, with adsorption including all possibilities except for a covalent bond. In the case of colloidal metals as direct markers, particularly in the case of colloidal gold, adsorptive bonds are preferably used.

[0025] According to the invention, it is essential that both conjugates are located in or onto the matrix material upstream the zone with an immobilised analyte or analyte analogue in such a way that they can be removed by fluid and can be transported into the direction of the detection zone.

[0026] According to the invention, both conjugates or even only one of the conjugates can be located upstream the sample application zone in the element of the invention. In this case, it is necessary that there is also an elution agent

application zone upstream the sample application zone. In this case, it is necessary that after applying a sample containing analyte onto the sample application zone, the conjugate(s) is (are) transported through the sample application zone in the direction of the detection zone by means of an elution agent which was specifically applied. Water or suitable aqueous solutions like buffers can be used as elution agents.

[0027] In an alternative embodiment of the element for analysis of the invention, no elution agent application zone which is separate from the sample application zone is provided for. This is sufficient if the sample application zone is located upstream the zone or zones with the conjugates, the zone with an immobilised analyte or analyte analogue and the detection zone.

[0028] It is also possible that upstream the sample application zone, there is an elution agent application zone either on separate matrix materials or on the same matrix material.

[0029] Using two conjugates in an element of the invention for determining an analyte as described above has considerable advantages vis-à-vis the embodiments of the state of the art. Thus, the universally usable conjugate 2 is a stable conjugate of a visually detectable marker and a binding partner with biological affinity having high sensitivity vis-à-vis a low-molecular organic molecule. Conjugate 2 can be manufactured reproducibly. By using a low-molecular organic molecule as detectable marker 1, it is possible to conjugate polyclonal antibodies or monoclonal antibodies by chemical methods simply and in the same quality in a reproducible manner. Products which are defined in that manner are manufactured. Commonly, in the case of immunoassays with marked binding partners of the analyte, the marked binding partner is a critical component. Work with respect to optimisation, in particular as regards the storability of the component on the element for analysis, but also already prior to processing and as regards the sensitivity of this component, does to a large extent not occur in the case of the present invention or is at least rendered considerably easier. In this context, reference is also made to the optimisation of the reaction conditions on the element for analysis and the ability to elute the conjugate in the element for analysis, which

can be largely standardised in that manner. These working steps are substantially merely limited to conjugate 1, which, however, due to the nature of its components, can be optimised considerably easier than a conjugate which, depending on the analyte, consists of changing binding partners with biological affinity and a visually detectable marker, which are often both heterogenic to a large extent and can be determined only in an insufficient manner and can thus, with respect to the product, be manufactured in the same, reproducible quality with difficulty only.

[0030] The determination of an analyte by means of an element of the invention is carried out in such a way that the sample which is to be examined for the presence of an analyte is contacted with the sample application zone. Either the analyte itself has already been dissolved in the fluid or suspended or additional fluid is applied onto the element as elution agent in order to move the ingredients of the sample, particularly the analyte which is possibly present, with the fluid in the direction of the detection zone. Thereby the analyte present in the sample will be contacted with the mixture of conjugates 1 and 2 and will react with them to form a detection complex. This detection complex is transported into the detection zone with fluid where it is determined. Only if analyte was present in the sample, can the visually detectable marker 2 in form of the detection complex mentioned earlier reach the detection zone and can be detected there. If no analyte was in the examined sample, the mixture of conjugates 1 and 2 will be bound in the zone with immobilised analyte or analyte analogue and no visually detectable marker 2 will reach the detection zone. In order to function in an optimal way, both conjugate 1 and conjugate 2 will be present in such a concentration, that the analyte or the analyte analogue immobilised between the sample application zone and the detection zone are capable of completely binding the conjugates. With respect to the analyte to be determined, the conjugates and correspondingly also the immobilised analyte or the analyte analogue should be present in excess. It is particularly preferred that conjugate 1 be present in excess with respect to conjugate 2 as thereby a particularly high sensitivity is achieved. A six to ten fold excess of conjugate 1 with respect to conjugate 2 has proven particularly advantageous.

[0031] If the sample is to be transported with an additional elution agent through the element of the invention into the detection zone, a kit consisting of the element for analysis of the invention and a corresponding elution agent has proved advantageous. In this context, the elution agent can be water or an aqueous solution, preferably a buffer solution, with the elution agent being in a corresponding container. This container can, for example, be a dropping bottle in order to apply the fluid onto the elution agent application zone. It can, however, also be a cup which is closed with a lid when not used but the lid of which can be removed for carrying out the determination process and an element of the invention can be placed into the cup containing the elution agent fluid in such a way that elution agent is absorbed via the elution agent application zone and passes through the different zones up to the detection zone.

[0032] Fig. 1 to 4 show a top view of four different possible embodiments of the element of the invention.

[0033] A particularly preferred embodiment of an element of the invention is shown in Figure 1. Matrix materials (1-4) are fixed next to each other in such a way onto a stiff, inert support sheet (5) that their front areas touch or slightly overlap. Matrix materials (1-4) represent the test zones of an element for analysis of the invention. Preferably, each consists of different absorbent materials (papers, fleeces, porous plastic layers and the like), whereby the fluid contact at the contacting edges is effected by placing the layers sufficiently close to each other. In an alternative embodiment it is, however, of course also possible to produce several zones which are located next to each other from the same material in one piece or in several pieces. On the whole, the test zones build a fluid transport zone leading from the sample application zone (1) via the conjugate zone (2) containing conjugate 1 and conjugate 2, and the recipient zone (3) containing immobilised analyte or analyte analogue, into the detection zone (4). After previous mixing of corresponding solutions or suspensions, conjugates 1 and 2 can be applied together in conjugate zone (2). Conjugate zone (2) can also first be impregnated with the conjugate and then again be impregnated with the other conjugate. Or the

conjugate zone (2) can contain two matrix materials lying onto one another which are identical or different and which each carry another conjugate.

[0034] The present case refers to an element for analysis where either so much fluid sample is applied that the fluid volume is sufficient in order to provide all matrix materials including the detection zone (4) with fluid or where first sample is applied onto the sample application zone (1) which is then transported through the element of the invention by a special elution agent which is also applied onto the sample application zone (1). In the case of a sample containing analyte when transporting the analyte with fluid through the different zones (1-4), starting with the conjugate zone (2) a detection complex is built from analyte and conjugates 1 and 2. This complex passes the recipient zone (3) and reaches the detection zone (4) where for example a gold marker used as marker 2 can be detected with the naked eye as red colouring. In the case of a sample which does not contain analyte, during the fluid transport through the zones (1-4) of the element of the invention, the conjugates are transported from the conjugate zone (2) to the recipient zone (3) where the conjugate mixture is bound to immobilised analyte or analyte analogue. In this case, visually detectable marker 2 will not reach the detection zone (4). No colouring will be detectable there.

[0035] Fig. 2 and 3 show elements of the invention where an elution agent application zone (6) precedes the sample application zone (1). If such elements are used, the sample is first applied onto the sample application zone (1). Subsequently, so much elution agent is applied onto the elution agent application zone (6) that analyte is transported into the conjugate zone (2) where a complex with conjugates 1 and 2 can be formed and that, via the zone (3) with an immobilized analyte or analyte analogue, the complex built reaches the detection zone (4) where the detection of the analyte is carried out.

[0036] After application of the samples, however, the elements of Fig. 2 or 3 can be placed in so much elution agent with the elution agent application zone (6) that in the case of an element according to Fig. 2, the sample application zone (1) or in the case of an element according to Fig. 3, the conjugate zone (2) is located above the fluid level of the elution agent.

[0037] While with respect to Fig. 2 the sequence of the zones (1-4) is the same as regarding the element according to Fig. 1, in the element according to Fig. 3, the sequence of the sample application zone (1) and the conjugate zone (2) is exchanged. Here, the elution agent is first contacted with conjugates 1 and 2 and carries out a pre-incubation before the analyte is contacted and bound.

[0038] Fig. 4 shows an element of the invention containing two zones 2a and 2b located next to each other, each carrying another of the two conjugates 1 and 2. Here, the conjugates can be located on the same matrix material arranged in one or in several pieces or they can be present on matrix materials of different kinds. The sequence of zones (1), (3) and (4) amongst each other and with respect to the conjugate zone which is split in partial zones 2a and 2b is identical to the one shown in Fig. 1. Thus, the function of the element according to Fig. 4 corresponds to the function described for the element according to Fig. 1 except for conjugates 1 and 2 being solved subsequently.

[0039] The invention is described in more detail by the following example.

Example 1

Determination of benzodiazepine by means of an element according to Figure 1

A. Preparation of a conjugate from gold and monoclonal antibodies against digoxin

[0040] Two conjugates are prepared. Conjugate A contains gold particles of an approximate size of 40 nm, loaded at an antibody concentration of 2 mg/l. Conjugate B contains gold particles of an approximate size of 20 nm, loaded at an antibody concentration of 10 mg/l.

[0041] Gold sol with an average particle diameter of approximately 40 nm or approximately 20 nm was prepared according to the method of Frens (Frens, G., "Preparation of gold dispersions of varying particle size: controlled nucleation for the regulation of the particle size in monodisperse gold suspensions" in Nature:

Physical Science 241 (1973), 20-22) by reduction of a 0.01 weight percent tetrachloride auric acid solution by heating with trisodiumcitrate.

[0042] The preparation of antibody-gold conjugate was carried out according to the method of Roth, J. "The colloidal gold marker system for light and electrone microscopic cytochemistry" in Bullock, G. R. and Petrusz, P., eds., "Techniques in Immunocytochemistry", Vol. 2, New York, Academic Press, 1983, 216-284.

[0043] After cooling of the gold sol solution described above to room temperature, the pH of the gold sol was adjusted with 0.2 M potassium carbonate solution to pH 8.0. A dialysed solution of a monoclonal IgG antibody against digoxin (Supplier: Roche Diagnostics GmbH, Mannheim, Germany) was added to the gold sol. Here, the volume ratio IgG-solution to colloidal gold solution was 1:10. After 30 minutes stirring at room temperature, the gold conjugate was saturated by adding a highly concentrated bovine serum albumin solution (end concentration in the conjugate solution: 1 mg/ml).

[0044] The gold conjugate was concentrated by ultrafiltration against a 20 mM Tris buffer pH 8.0 to an optical density of typically 20 (Extinction at 525 nm and 1 cm light path. The conjugate solution was finally filled up to 100 µm Brij® and 0.05 weight percent of sodium azide.

B. Production of conjugate from digoxin and polyclonal antibody against benzodiazepine

[0045] Sheep are immunised as in Example 1 of EP-A 0726275 with 7-chloro-3[2-(N-maleinimido)ethyl]oxy-1-methyl-5-phenyl-1H-1,4-benzodiazepine-2(3H)-on the synthesis of which is known from Example 3 of EP-A 0726275. 100 ml serum of a correspondingly immunised sheep containing approximately 6.5 g protein were first treated with 1.5 g aerosil (1 hour at room temperature) and centrifuged. The supernatant was decanted and was adjusted to 1.9 M with ammonium sulfate. Thereby, the IgG was precipitated. The precipitate was centrifuged and the supernatant was removed. The precipitate was taken up with weak PBS buffer pH 7 and dialysed. The dialysate was negatively purified over 100 ml DEAE-Sephadex

ff. In this process, impurities remained on the column and the immunoglobulins passed through. The passage was collected (detection at 280 nm) and rebuffered in PBS buffer pH 7.4 for the immunsorption. The yield was 3.5 g IgG in 90 ml solution.

[0046] 3.5 g IgG in 90 ml from the DEAE purification was pumped over 50 ml immunoadsorber (Spherosil to which a polyhapten from Temazepam on rabbit IgG (see Example 1D) is bound) in circulation. In this process, the immunospecific IgGs bound and all other proteins passed through. Another wash was effected with PBS buffer. The bound IgG was also washed with two column volumes sodium chloride/Tween 20 and two column volumes 30 mM sodium chloride. Then a stepwise elution was carried out. The first step is the elution with 3 mM hydrochloric acid at 4 to 8 °C, the second step is the elution with 1M propionic acid at 4 to 8 °C and the third step is the elution with 1M propionic acid at room temperature. The eluates were immediately dialysed against ice water. The eluates were additionally dialysed against 1 mM acetic acid and after being concentrated they were filtered and lyophilised. The yield amounted to approximately 300 mg polyclonal IgG antibodies against benzodiazepine.

[0047] The polyclonal antibodies produced in such a way were dissolved in water so that a concentration of 12 mg antibodies per ml was adjusted. It was re-buffered with 1.5 ml 1 M potassium phosphate buffer pH 8.3 to 0.1 M potassium phosphate and adjusted to 10 mg antibodies per ml. Digoxin-succinimide ester was dissolved in DMSO to a concentration of 10 mg/ml and a 6-fold molar excess of the digoxin derivative was pipetted at 4 °C into the antibody solution. It was left to react for three hours at 4 °C. Subsequently, a dialysis was carried out against the 50-fold volume of 20 mM Tris pH 8 and against the 50-fold volume of 50 mM sodium chloride, respectively, for at least four hours each.

C. Production of polymerised streptavidin

[0048] Polymerised streptavidin was produced as described in Examples 1c and 1d in EP-B 0331127.

D. Production of biotinylated Temazepam polyhapten

[0049] 1.2 g unspecific, freeze-dried polyclonal rabbit IgG was dissolved in 40 ml potassium phosphate buffer pH 8.5 and after centrifugation, the clear supernatant was decanted off. The supernatant contained 950 mg protein. 12.4 mg S-acetylthiopropionic acid succinimidylester were dissolved in 1.24 ml DMSO, added to rabbit IgG solution and stirred for two hours at room temperature. The reaction was stopped by adding 0.5 ml 1M lysine solution and dialysed against triethylammoniumcitrate/EDTA solution pH 6.5.

[0050] After dialysis, the activated rabbit immunoglobulin was deacetylated with 1 ml 1M ammoniumhydroxide solution per 25 ml immunoglobulin solution at pH 6.5 for two hours at 4°C.

[0051] For 950 mg deacetylated activated rabbit IgG in 50 ml buffer, 10.8 mg Temazepam (production according to Example 3 in EP-A 0726275) were dissolved in 1.08 ml DMSO and added to the deacetylated activated rabbit immunoglobulin solution. After two hours at 4°C, the reaction was stopped with 2 ml 0.1 M cysteine and the solution was incubated for 30 minutes with 2 ml 0.5 M iodine acetamide and subsequently dialysed against triethanolamine buffer pH 8.5 for 30 minutes at room temperature.

[0052] 24 mg biotin succinimidylester, dissolved in 2.4 ml DMSO, were added to the 950 mg Temazepam polyhapten produced in said manner and stirred for two hours at room temperature. Subsequently, an intensive dialysis against weak acetic acid took place (10 to 1 mM). After the dialysis, the dialysate was adjusted with 1M sodium acetate pH 4.2 to 20 mM sodium acetate pH 4.2 and purified via Tris-acryl-carboxymethylcellulose by means of column chromatography.

E. Production of elements of analysis according to Figure 1

[0053] 2 elements of analysis with different conjugate zones (2) according to the invention were produced.

[0054] The following zones according to Figure 1 were glued to carrier foil (5) with a breadth of 5 mm.

Sample application zone (1): polyester fleece by the company Binzer, Hatzfeld, Germany. A pure polyester fleece compacted with 10% Kuralon, exhibiting a thickness of 1.0 to 1.2 mm and having an absorptive capacity of 1,800 ml/m^2

conjugate zone (2): A mixture fleece of 80 parts polyester and 20 parts viscose staple fibre each, compacted with 20 parts Kuralon, having a thickness of 0.32 mm and an absorptive capacity of 500 ml/m^2, was soaked with one of the following solutions and dried:

- soaking solution A

  A mixture of 1 ml of the gold conjugate A produced in A. each, diluted in Hepes buffer (200 mM, pH 7.5) to an antibody concentration of 0.3 nmol/ml, and 1 ml of the digoxin conjugate produced in B., diluted in Hepes buffer (200 mM, pH 7.5) to a digoxin concentration of 2 nmol/ml, was incubated for one hour at room temperature. Subsequently, soaking the fleece takes place.

- soaking solution B

  A mixture of 1 ml of the gold conjugate B produced in A. each, diluted in Hepes buffer (200 mM, pH 7.5) to an antibody concentration of 1.4 nmol/ml, and 1 ml of the digoxin conjugate produced in B., diluted in Hepes buffer (200 mM, pH 7.5) to a digoxin concentration of 2 nmol/ml, was incubated for one hour at room temperature. Subsequently, soaking the fleece takes place.

trapping zone (3): A fleece of 100% linters, compacted with two weight percent Etadurin having a thickness of 0.41 mm and an absorptive capacity of 386 ml/m^2 is soaked with a solution of 200 mg/l of the polymerised streptavidin produced in C in 50 mmol/l sodium phosphate pH 8.0 and is dried subsequently. Then, the pre-soaked fleece is soaked again with a solution of 300 mg/l of the biotinylated Temazepam polyhapten produced in D. in 50 mM/l sodium phosphate pH 8.0 and dried.

detection zone (4): A fleece of 100% linters, compacted with two weight percent Etadurin having a thickness of 0.35 mm and an absorptive capacity of 372 ml/m^2 is used.

F. Carrying out the test

[0055] For the determination of benzodiazepine by means of an element produced in E., the test strip is immersed into the fluid to be analysed for about 5 seconds so that approximately three quarters of the sample application zone (1) is below the fluid level. Then, the element is placed on a non-absorbing material horizontally. When the fluid front has completely wetted the detection zone (4) (in the case of aqueous solutions generally after two minutes at the most), a pink colouring shows the presence of benzodiazepines in the sample to be analysed. In the absence of substances that are recognised by the digoxin-marked antibody, the detection zone stays white. The intensity of the pink colouring correlates with the analyte concentration. A colour chart simplifies classification.

colour field (FF) 0: negative, no analyte
colour field (FF) 1: positive, light pink
colour field (FF) 2: strong positive, strong red colouring

Table of results					
	aqueous solution with Bromazepam		urine with Bromazepam		
	0 ng/ml	100 ng/ml	0 ng/ml	50 ng/ml	100 ng/ml
gold conjugate A	FF 0	FF 1-2	FF 0	FF 0-1	FF 1-2
gold conjugate B	FF 0	FF 2	-	-	-

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CLAIMS

1. Element for the determination of an analyte in a fluid by means of a specific binding reaction of two binding partner with biological affinity

   containing in or on the material allowing the transport of fluid between zones a sample application zone (1) and a downstream detection zone (4)

   and a zone (3) with an immobilised analyte or analyte analogue which is located between the sample application zone (1) and the detection zone (4)

   and upstream zone (3) with an immobilised analyte or analyte analogue, conjugate 1 which is impregnated and can be removed by a fluid and consists of a binding partner 1, which has biological affinity and is able to form a specific binding reaction with the analyte to be determined, and a detectable marker 1,
   characterised in that

   the detectable marker 1 is a low molecular organic molecule

   and upstream zone (3) with an immobilised analyte or an analyte analogue, a universal conjugate 2 is present which, too, can be removed by a fluid and consists of a binding partner 2, which has biological affinity and is able to form a specific binding reaction with the detectable marker 1, and a visually detectable marker 2.

2. Element according to claim 1, characterised in that the detectable marker 1 is digoxigenin or digoxin.

and is determined there.

11. Method according to claim 10, characterised in that the fluid is a sample fluid with the help of which the analyte is applied to the element.

12. Method according to claim 10, characterised in that in order to move the analyte, additional elution agent is applied to the elution agent application zone (6) according to claim 6.

13. Use of an element according to claims 1 to 9 for the determination of an analyte.

14. Kit for the determination of an analyte containing an element according to claims 1 to 9 and an elution agent.

Fig. 1

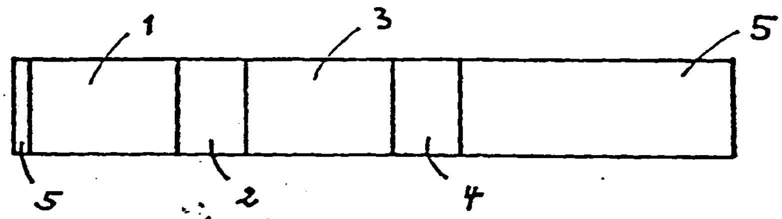

Fig. 2

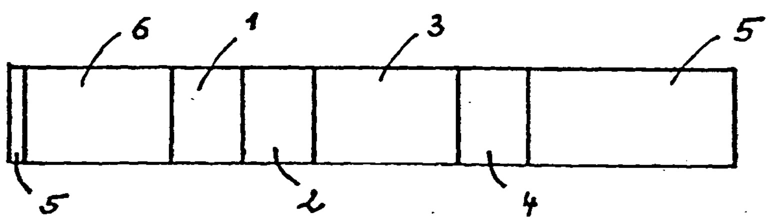

Fig. 3

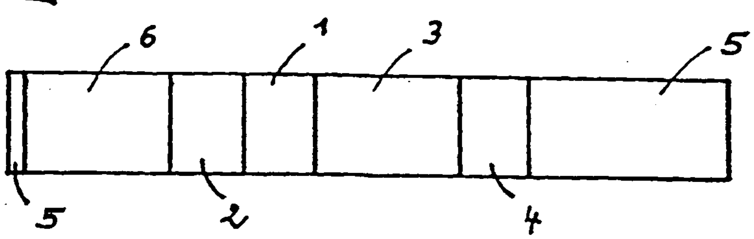

Fig. 4